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Vol NO 10/3

314596

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VACCINE THERAPY FOR PROSTATE CANCER

Benjamin A. Tjoa, PhD, Abdel-Aziz A. Elgamal, MD, PhD, and Gerald P. Murphy, MD, DSc

Current standard treatments for early-stage, localized prostate cancers are radical prostatectomy and radiotherapy. These procedures exhibit failure rates of more than 20%.[24] As a result, an increasing number of treated patients either manifest metastatic disease or are at high risk for the development of such a state. The options for these primary treatment failures and for metastatic cases, including hormonal, chemotherapeutic, and radiation strategies, are limited in terms of duration and efficacy.[17] Immunotherapeutic approaches to cancer treatment have shown some promise in experimental studies. Several immunotherapies are now being tested in clinical settings.

CANCER IMMUNOTHERAPY STRATEGIES

The human immune system has components that can recognize and kill tumor cells. The role of the immune system in tumor surveillance was initially suggested by the direct association between immunosuppression and the increased incidence of cancer.[23] In addition, leukocytes are often present surrounding and infiltrating tumor tissue.[36, 40] These tumor-infiltrating leukocytes have been isolated and characterized from various tumor tissues. T cells comprise a majority of leukocytes that infiltrate tumor tissues.[19, 27, 40]

T cells fall into two major categories: (1) the CD8+ cytolytic T lymphocytes and the CD4+ T helper cells. Both T-cell subsets have been shown to have a role in tumor regression in experimental systems.[50] Cytolytic T lymphocytes and T helper cells can recognize small peptides that are bound to class I or II major histocompatibility complex (MHC) molecules, respectively.[9] The MHC molecule is also known in the human system as the human leukocyte antigen (HLA). Cytolytic T lymphocytes isolated and cultured from tumor tissues specifically recognize peptides bound to class I HLA molecules on the tumor cell surface. These peptides are digestive products from proteins expressed by the tumor cells. Upon specific recognition of the peptide-HLA complex, cytolytic T lymphocytes are capable of killing the original tumor cells.[19, 27]

Early attempts in cancer immunotherapy focused on the in vitro expansion of tumor-

This work was supported in part by grants from CaP CURE Foundation, Phi Beta Psi Sorority, The Rontell Foundation, and Northwest Biotherapeutics, Inc.

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infiltrating leukocytes. Infusions of tumor-infiltrating leukocytes to patients with cancer, such as melanoma and renal carcinoma, have had limited success.[4, 11] Infusions of cytokines that enhance T-cell response, such as interleukin-2 and interferon-γ as systemic adjuvants, have also been used as techniques in cancer immunotherapy.[3, 39]

CANCER VACCINES: IN VIVO GENERATION OF ANTICANCER IMMUNE RESPONSE

An alternative to systemic cytokines and adoptive immunotherapy is the vaccination of patients to elicit in vivo activation of tumor-specific T cells. Irradiated tumor cells derived from the patient (autologous) or from other individuals (allogeneic) have been used to inoculate patients with cancer in the hope of generating a therapeutic immune reaction.[43]

Discoveries of cancer antigens and the genes that code for them have provided well-defined targets for T-cell attack.[51] Cancer antigens can be classified into two groups. The first group, cancer-specific antigens, are expressed only by cancer cells. These are often identified as products of mutated oncogenes or tumor suppressor genes (e.g., mutated ras or p53 peptides).[51] The second group, known as cancer-associated antigens, are present not exclusively in a particular neoplastic tissue. In some cases, these antigens are also expressed by their associated normal tissues (tissue-specific antigens) or by other cancer tissues. Three examples of prostate-associated antigens are prostate-specific antigen (PSA), prostatic acid phosphatase, and prostate-specific membrane antigen (PSMA).[15, 44, 53] Antigens shared by prostate cancer and other neoplastic tissues include mucins (MUC1 and 2), carcinoembryonic antigen, beta chain of human chorionic gonadotropin, HER2neu, and Thompson-Friedenreich antigen.[44, 55] Clinical trials have administered several of these antigens, including MUC1 and carcinoembryonic antigen.[44] Preliminary results suggest high titers of specific antibodies in response to immunizations.[44]

Because T cells recognize peptide antigens bound to HLA molecules, it is of interest to identify these peptide/HLA complexes that form the T-cell epitopes. With the recent availability of known motifs of HLA binding, it has been possible to predict from the amino acid sequence of tumor antigens the composition of short peptides with high affinities for certain HLA molecules.[9]

Recent immunologic studies have uncovered more details of the initiation of a T-cell response. This knowledge has provided a powerful tool to manipulate steps in T-cell activation to enhance the efficacy of a cancer vaccine. T-cell immune responses begin with the interaction of the T-cell receptor with antigenic peptides bound to HLA molecules expressed on the surface of specialized antigen-presenting cells.[26] If this interaction is accompanied by binding of costimulatory receptors (e.g., CD28) to their ligands (CD80 and CD86), an intracellular cascade of biochemical events is triggered, which results in T-cell activation and proliferation.[16, 54] Activated T cells can lyse cells that express the stimulating antigen and the appropriate MHC antigen, in this case, tumor cells. Additional factors that influence the activation and proliferation of T cells include CD40-CD40 ligand interactions and various cytokines (e.g., interleukins-2, -4, -10, and -12; granulocyte-macrophage colony-stimulating factor [GM-CSF]; and interferon-γ).[50] The presence or absence of these cytokines can have a profound influence on the potency and character of the immune response. Some procedures have introduced genes coding for cytokines or costimulatory molecules into tumor cells prior to inoculation to enhance recognition by the immune system.[35]

Efforts are underway to develop genetically engineered vaccines for prostate cancer. Ongoing preclinical studies are attempting to introduce cytokine genes into prostate tumor cell lines.[44] Human trials at Johns Hopkins have involved transfection of tumor cell lines established from prostate cancer at the time of prostatectomy with the GM-CSF gene for in vivo administration.[44] The success of these autologous and HLA-restricted vaccines has been limited by the feasibility of growing the prostate cancer cell lines from each patient.[44]

The most recent advance in cancer vaccines

has been the use of autologous antigen-presenting cells to present tumor antigens to T cells.[27, 45] Antigen-presenting cells are members of the hematopoietic cell family that have unique capabilities to present antigens to T cells.[22] The rationale for using these cells is that all of the factors necessary for initiation of the immune response, including those not yet defined, are already present.[50]

DENDRITIC CELLS: A VEHICLE FOR CANCER VACCINE DELIVERY

Dendritic cells (Fig. 1) are considered the most potent antigen-presenting cells of the immune system.[45] Dendritic cells were originally identified as a cell type with unique dendritic morphology and highly efficient antigen-presenting capabilities. They have unique antigen uptake and migratory capacities.[1, 45] Dendritic cells lack most of the markers typical of other leukocyte populations (e.g., CD3, CD14, CD15, CD16, and CD19, which are markers for T cells, macrophage/monocytes, granulocytes, natural killer, and B cells, respectively).[45] They express high levels of both class I and class II HLA molecules. Dendritic cells also express one or both of the costimulatory molecules CD80 and CD86.[45] In addition, dendritic cells express cell adhesion molecules and CD40 and produce cytokines such as IL-12, which are involved in T-cell activation.[45, 50] Figure 2 depicts some of the well-characterized T cell–dendritic cell interactions during a T-cell activation event. Although other antigen-presenting cells such as B cells and macrophages are also effective stimulators of memory T cells, dendritic cells are unique in their ability to stimulate naïve T cells. Thus, it is well accepted that dendritic cells have an important role in driving an initial T-cell response against tumor cells.[50]

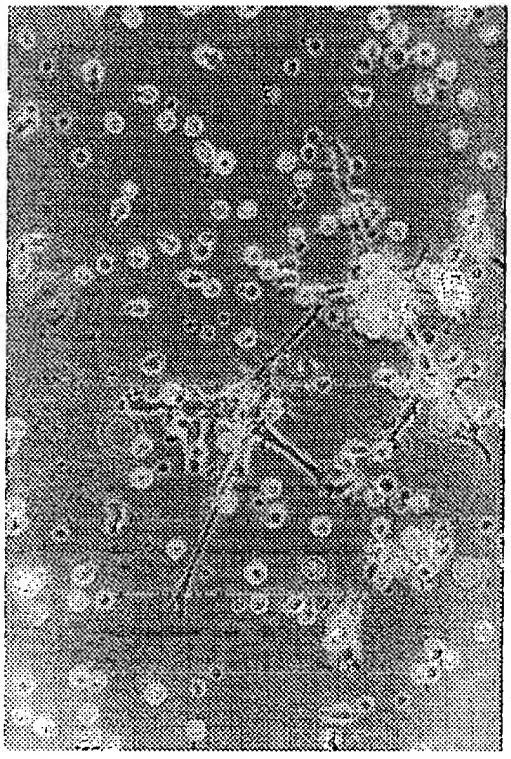

Figure 1. Morphology of dendritic cells. Dendritic cells with long extended processes are shown in clusters with lymphocytes (small cells). These cells were isolated from a lymph node, cultured in the presence of GM-CSF and IL-4. (Original magnification × 400.)

Dendritic cells are found in the epidermal layer of the skin, the respiratory and gastrointestinal systems, and the interstitial regions of several solid organs where they function as sentinels, capturing invading microorganisms for presentation to immune cells.[1] Until recently, the use of dendritic cells for cancer vaccine studies was limited because few cells could be isolated from tissues or peripheral blood.[21, 45] With improvements in isolation and culture, much larger numbers of dendritic cells are available, and immunotherapy using dendritic cells is now feasible.[5, 6, 38, 47]

Dendritic cells can be isolated directly from blood using a cocktail of monoclonal antibodies.[37] Alternatively, they can be cultured from CD34+ progenitor cells collected from either the bone marrow or cord blood.[6] In addition, dendritic cells can be generated by culturing adherent peripheral blood monocytes in the presence of GM-CSF and interleukin-4 (IL-4).[5, 38, 47]

The efficacy of dendritic cell presentation of tumor-associated antigens in humans has been reported. Specific cytolytic T lymphocytes have been generated from dendritic cells pulsed with several melanoma-associated antigens, such as MART-1, gp100, and tyrosinase, as well as prostate-associated anti-

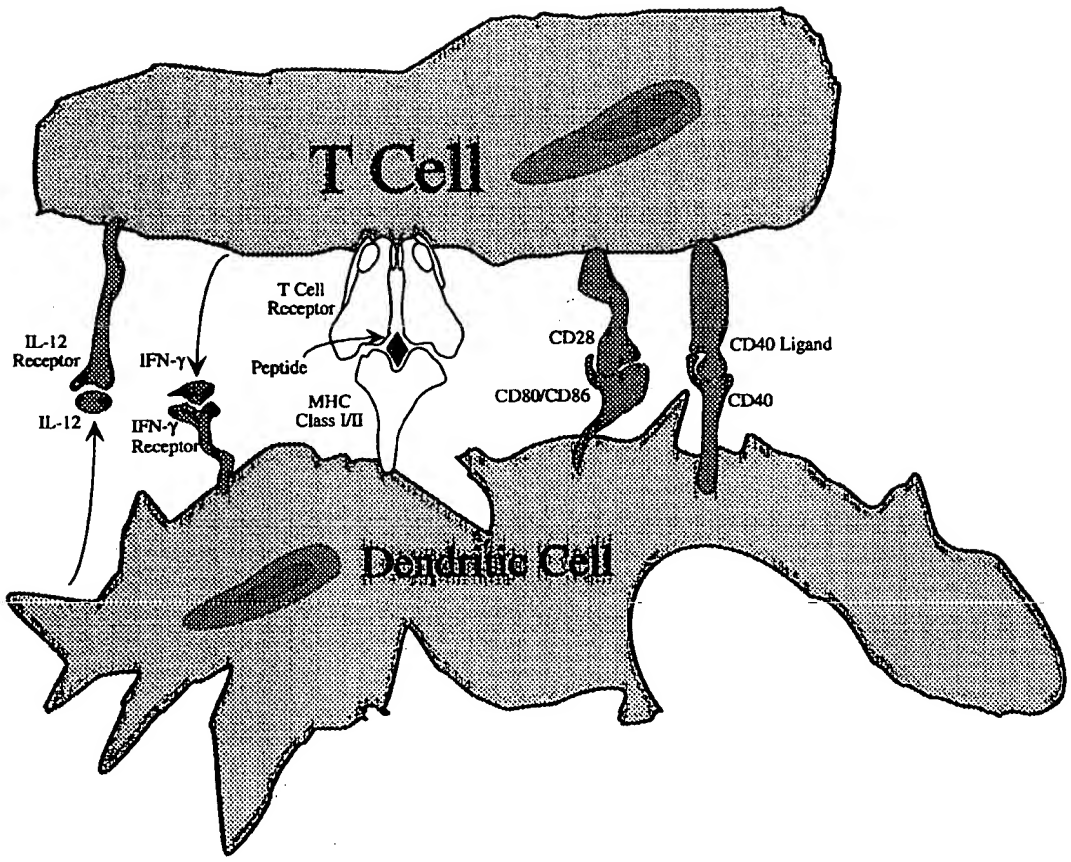

Figure 2. Interactions between a T cell and a dendritic cell involved in T cell activation. T-cell receptor recognition of peptide antigen/MHC product is shown. In addition, binding of costimulatory molecules and their ligands, and certain cytokines and their receptors are shown.

gens, such as an autologous prostate tumor cell lysate and PSMA.[2, 46, 52]

Dendritic cell–based cancer vaccines have been tested with some success in clinical trials of patients with several types of cancers, including follicular B lymphoma and melanoma.[14, 29, 34] The authors have been developing a prostate cancer vaccine using autologous dendritic cell as a vehicle to present prostate antigens to T cells in vivo. Phase I and II clinical trials of dendritic cells pulsed with HLA-A0201 PSMA peptides are described herein.

PROSTATE CANCER VACCINE

There are two major components of the prostate cancer vaccine developed by the authors. The first component is dendritic cells, which are isolated and cultured from patients' peripheral blood. The second component is a specific antigen used to target prostate cancer tissues. Current immunotherapy protocols use PSMA as the target antigen for T-cell attack in vivo.

Prostate-specific membrane antigen is a 750 amino acid membrane-bound protein expressed by prostate epithelial cells.[15] One of the monoclonal antibodies specific for PSMA (7E11-C5.3) is used in a prostate cancer imaging method (ProstaScint, Cytogen Corp, Princeton, NJ).[13] Elevated expression of PSMA has been detected in hormone-refractory prostatic carcinoma.[18] In addition, levels of PSMA are elevated in the serum of patients with advanced hormone-refractory prostate cancer.[30] Using HLA-specific motifs, the au-

thors identified PSMA peptides with a high affinity for a class I HLA molecule expressed by a large fraction of the population (HLA-A0201). Two of these peptides (PSM-P1 and PSM-P2) were used as antigens in phase I and II clinical trials.[32, 48, 49] Figure 3 shows the approximate location of the two peptides within the PSMA molecule.

PHASE I CLINICAL TRIAL

The phase I study was conducted at Pacific Northwest Cancer Foundation, Northwest Hospital, in Seattle, Washington.[32] It examined the administration of HLA-A0201–specific PSMA peptides (PSM-P1 and PSM-P2), autologous dendritic cells, and PSM-P1 and P2 pulsed autologous dendritic cells to 51 patients with advanced hormone-refractory prostate cancer. The majority of these patients (39 of 51) were in stage D2 (T4N1-3M1a-c) according to the American Joint Committee on Cancer staging system. Many of them were anemic and had undergone various treatments that resulted in an impaired immune competency. Less than 25% of the population were considered fully immunocompetent at the start of the study as assessed by delayed-type hypersensitivity skin tests.

All participants received four infusion cycles of the test substances over 6-week intervals. The number of infusion cycles and the interval between cycles were determined to optimize the generation of immune response to the test substance. At the completion of four cycles of infusions, the maximum tolerated dose was not achieved. Neither significant acute nor chronic toxicity was observed in all doses of test substances, except for mild-to-moderate cases of hypotension without pulse change during the time of infusion. In addition, no significant increase in serum tumor necrosis factor alpha or interferon-γ was measured during the course of the study.[32]

Patients were monitored for cellular immune modulation to the appropriate PSMA peptides (PSM-P1 or P2). An increased cellular response was observed within the HLA-A2–positive subjects who were infused with dendritic cells pulsed with PSM-P1 or P2.[32] Patient clinical response was analyzed based on National Prostate Cancer Project (NPCP) criteria plus a minimum of 50% reduction of serum PSA levels. Seven partial responders were observed.[32, 48] Average PSA levels showed an increase in the nonresponder group, whereas a decrease was observed in the seven partial responders (Fig. 4).

PHASE II CLINICAL TRIAL: ASSESSMENT OF EFFICACY OF ADMINISTRATION OF AUTOLOGOUS DC/PSM-P1 AND P2 PEPTIDES

The authors' phase II trial started in January 1997 and enrolled 107 subjects. Partici-

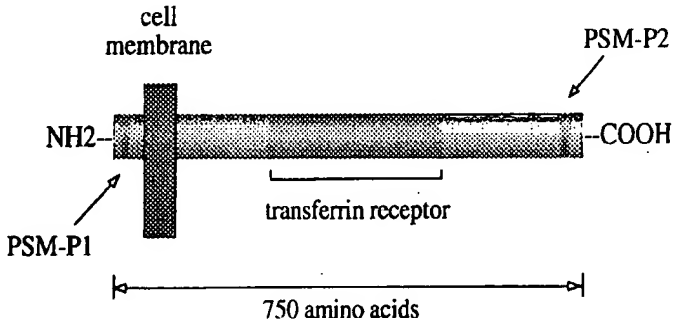

Figure 3. Prostate-specific membrane antigen (PSMA). PSMA molecule is depicted showing the intracellular, transmembrane and extracellular domains. A region with high homology to human transferrin receptor, and two regions corresponding to the two HLA-A0201 peptides (PSM-P1 and PSM-P2) are shown.

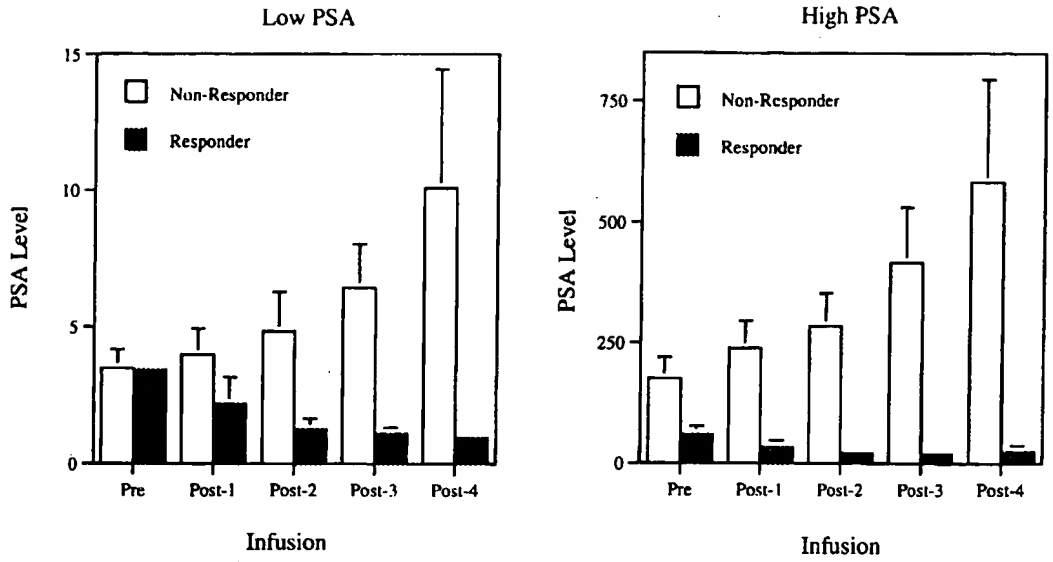

Figure 4. PSA levels of responder and nonresponder populations. Each group is divided into low (initial PSA values 0–19) and high PSA (initial PSA values greater than 19) categories. Normal ranges for PSA are 0 to 4 ng/mL. Mean and standard error of the mean (SEM) values were obtained from patient sera drawn pre-infusion and 7 days postinfusion. There were 24 patients in the nonresponder and two patients in the responder low PSA groups. There were 20 patients in the nonresponder and five patients in the responder high PSA groups. In the latter, the mean PSA pre-infusion for the nonresponder was 175.0 ng/mL, and at the end of four infusions, it was 583.4 ng/mL. For the responder group, the mean PSA values were 60.0 ng/mL at the pre-infusion level and 24.4 ng/mL at the completion. In both groups, significant differences in pre- versus post-infusion values (*P* value less than 0.05) were observed. (*From* Tjoa B, Erickson S, Bowes V, et al: Follow-up evaluation of prostate cancer patients with autologous dendritic cells pulsed with PSMA peptides. Prostate 32:272, 1997; with permission.)

pants in this study included 66 patients with hormone-refractory metastatic prostate cancer (group A). Half of group A participants (33 of 66) were participants in the previous phase I study who had requested to be enrolled in the phase II trial. Group B included 41 patients with evidence of local recurrence after failure of a primary treatment (e.g., slowly rising PSA, positive prostate biopsy, or detection of pelvic lymph node on a ProstaScint scan).

Study participants received a total of six infusions of autologous dendritic cells pulsed with a PSM-P1 and P2 cocktail at 6-week intervals. With each infusion, half of the study subjects received a 7-day course of subcutaneous injection of GM-CSF as systemic adjuvant. At the conclusion of the scheduled infusions and follow-up observations, clinical status was evaluated based on NPCP criteria and 50% reduction of PSA.

A total of 33 patients with metastatic prostate cancer who participated in the phase I study have completed the phase II study. Based on the NPCP criteria and 50% reduction in PSA, 9 of the 33 subjects (27.3%) were identified as partial responders.[49] Eleven patients (33.3%) exhibited no significant change during the phase II trial. Thirteen patients exhibited disease progression. Seven patients died during the study (Table 1).

Among the nine partial responders, four were also responders in the phase I study. Four of the five patients who were nonresponders in the phase I study were in treatment groups that did not receive autologous dendritic cells pulsed with PSM-P1 or P2. A majority of the partial responders exhibited improvements in general immune response as assessed using delayed-type hypersensitivity skin test to recall antigens. This phase I/II study encompassed an average total period of 613 days. Twelve of nineteen subjects (63%) with stage D2 hormone-refractory metastatic

Table 1. CLINICAL STATUS OF PHASE II CLINICAL TRIAL SUBJECTS BASED ON NPCP CRITERIA PLUS 50% REDUCTION IN PSA

Clinical Status	Number of Patients (%)	HLA-A2+(−)	Stage (TNM Stage)		
			D0 (T4N0M0)	D1 (T4N1–3M0)	D2 (T4N1–3M1a–c)
Progression	13* (39.3%)	12 (1)	2	1	10
No change	11 (33.3%)	5 (6)	5	2	4
Partial response	9† (27.3%)	6 (3)	3	1	5
TOTAL	33 (100%)	23 (10)	10	4	19

*Six patients from this group are deceased.
†One patient from this group is deceased.
From Tjoa BA, Simmons SJ, Bowes VA, et al: Evaluation of phase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides. Prostate 36:41, 1998; with permission.

prostate cancer survived a period of over 600 days (median survival period, 608 days). This is a significant observation in comparison with other studies of similar populations with hormone-refractory metastatic prostate cancer, which have exhibited a median survival of 6 months.[20] The remaining study participants will be evaluated for response after all scheduled infusions are completed.

RESPONSE MONITORING IN DENDRITIC CELL VACCINE THERAPY

All study participants should be evaluated before, during, and after treatment for both clinical and immunologic responses. Periodic measurement of PSA, bone alkaline phosphatase, complete blood counts, and radiographic imaging are essential. Monitoring treatment responses to vaccine therapies has not been an easy task because patients with metastatic prostate cancer have a history of hormone therapies and dedifferentiated tumors. There is a lack of correlation between PSA levels and the presence of metastases in hormone-refractory prostate cancer.[10] Measurement of PSMA serum levels could be an alternative biochemical marker that is not influenced by tumor dedifferentiation or hormone therapy.[8] Radiodiagnostic imaging has provided another valuable modality for the identification of measurable soft-tissue or bony metastases. Bone scan and radiographs, CT, and ProstaScint scintigraphy scan have been accepted for the detection of occult metastases months before the appearance of related manifestations or symptoms.[7, 33]

In addition, the authors have designed a battery of in vitro clinical tests to assess and monitor immune response to vaccine therapy.[41] The frequency of antigen-specific cells is expected to be low, ranging from 1 in 100,000 to 1 in 1,000,000 T lymphocytes prior to immunization. Following the infusion of peptide-pulsed dendritic cells, the frequency of antigen-specific T cells may increase tenfold. Detection and quantitation of these low-frequency cells requires sensitive assays of T-cell activity. The number of peptide-specific T cells is quantitated in an ELISPOT assay based on their secretion of interferon-γ following in vitro stimulation with peptides.[12, 42] The amount of interferon-γ secreted following stimulation with peptides is also measured as is secretion of interleukin-10 (IL-10). Secretion of interferon-γ indicates the development of type 1 T cells, which are thought to represent the type of immune response required for antitumor effects. IL-10 is considered a regulatory cytokine produced by type 2 T cells. IL-10 has been shown to suppress antigen presentation by dendritic cells and to inhibit the secretion of cytokines by type 1 T cells. Therefore, IL-10 secretion is monitored to detect immune responses that might inhibit antitumor effects. Cytokine secretion assays are performed on primary cultures of peripheral blood lymphocytes directly following isolation from peripheral blood and also after several rounds of in vitro stimulation. Repeated in vitro stimulation will increase the number of antigen-reactive cells to the level where they can be more readily detected. In addi-

tion, the T-cell receptor-zeta chain expression within the CD3+ and CD16+ populations from patient peripheral blood mononuclear cells is also analyzed using flow cytometry. At the conclusion of the authors' phase II study, intergroup study comparison, both clinical and immunologic, will be completed.

FUTURE DIRECTION

Studies performed to date support the use of autologous dendritic cells as a vehicle to deliver specific target antigen in prostate cancer vaccines. In a future clinical trial, the authors will expand the antigen repertoire of vaccines from two HLA-A0201–specific PSMA peptides (each of which represents nine amino acids) to a recombinant PSMA protein consisting of the native sequence without the transmembrane domain. Table 2 summarizes the five treatment arms in this clinical trial.

A total of five distinct treatment arms will be administered in this clinical trial. Groups 1 and 2 will receive recombinant PSMA alone or in alum, respectively. Group 3 will receive cultured autologous dendritic cells pulsed with recombinant PSMA. Group 4 will receive subcutaneous injection of GM-CSF as a systemic adjuvant in addition to infusion of cultured autologous dendritic cells pulsed with recombinant PSMA. Group 5 will initially receive a course of injection of Flt3 ligand, a potent enhancer of hematopoietic differentiation from several lineage precursors including dendritic cells.[25] In these patients, dendritic cells generated in vivo by Flt3 ligand administration will be used as the antigen-presenting cells. The highest patient-tolerated dose, acute/chronic clinical response, as well as T-cell–mediated immune response will be determined in this clinical trial. The discovery of new antigens as well as the optimization of dendritic cell culture, loading, and delivery currently being conducted at the authors' center are key to developing future generations of prostate cancer vaccines.

Table 2. FIVE TREATMENT ARMS IN THE PHASE I CLINICAL TRIAL OF PATIENTS WITH METASTATIC HORMONE-REFRACTORY PROSTATE CANCER USING AUTOLOGOUS DENDRITIC CELLS AND RECOMBINANT PROSTATE CANCER MEMBRANE ANTIGEN

1. Subcutaneous injection of recombinant PSMA (0.1, 1.0; or 10.0 μg) in saline
2. Subcutaneous injection of recombinant PSMA (0.1, 1.0; or 10.0 μg) in alum
3. Intravenous infusion of cultured autologous DC + PSMA (0.1, 1.0, or 10.0 μg)
4. Intravenous infusion of cultured autologous DC + PSMA (0.1, 1.0, or 10.0 μg) and subcutaneous injection of GM-CSF as systemic adjuvant
5. Subcutaneous injection of Flt3 ligand + intravenous infusion of in vivo generated autologous DC + PSMA (0.1, 1.0, or 10.0 μg)

DC = dendritic cells; PSMA = prostate-specific membrane antigen; GM-CSF = granulocyte-macrophage colony-stimulating factor.

ACKNOWLEDGMENT

The authors thank the staff at Northwest Hospital Day Surgery/Short Stay Unit, expecially Nancy Martin, Sharon Vitolo, Frances Seemann, and Lanelle Bentz.

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